THE DNA REPEAT LENGTHS IN CHROMATINS FROM SEA URCHIN SPERM AND GASTRULA CELLS ARE MARKEDLY DIFFERENT

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1. Introduction

We have shown that the DNA repeat length in the chromatin of higher eukaryotes is variable [1]. In view of the recent discovery of the existence of variants of histones H2A and H2B in animal cells [2–5], we have suggested among other possibilities, that this variability could be related to the existence of different histone cores in different cells depending on the histone variants which are present. Sea urchin cells are particularly attractive to study such a possibility, since the existence of multiple forms of histones H2A and H2B have been demonstrated during embryogenesis [2] and histone H2B is replaced by a related fraction in sperm cells [6,7]. Furthermore, histones H3 and H4 are not acetylated in sea urchin sperm cells, whereas they are multiacetylated during embryogenesis at the gastrula stage [8]. Multiple forms of histones H1 have also been found in sea urchin cells during embryogenesis [9–11] and a very basic variant of histone H1 is present in sperm cells [6]. We report here that the DNA repeat lengths of chromatins from sea urchin sperm and gastrula cells are markedly different, but both higher than that of rat liver chromatin.

2. Materials and methods

Eggs and sperm cells were collected separately from *Arbacia lixula* sea urchins by shaking gonads in artificial sea water. Eggs were filtered through cheese cloth, washed with artificial sea water and fertilized with a concentrated suspension of sperm. The embryos were grown at 20°C at a concentration of 3000–4000 per ml, and collected at the gastrula stage (about 24 h after fertilization). Nuclei were then prepared according to Burgoyne et al. [12]. Nuclei from sperm cells were prepared according to Mizuno [13] with some modifications. Sperm cells were washed once in a buffer containing 0.25 M sucrose, 50 mM Tris–HCl pH 7.5, 2 mM $MgCl_2$ and 1% Triton X-100 (Buffer A), and then three times in the same buffer but without Triton (Buffer B). Sperm heads were purified by centrifugation (14 000 × *g*, 10 min) through a solution containing 2 M sucrose, 50 mM Tris–HCl pH 7.5 and 2 mM $MgCl_2$. The pellets of embryo nuclei and of sperm heads were suspended in the nuclease digestion buffer at a concentration of DNA about 1 mg/ml. In some experiments the purified embryo nuclei were submitted to the same treatment as the sperm heads. In brief, they were suspended in buffer A and then washed three times in buffer B.

Nuclease digestion was carried out on nuclei with micrococcal nuclease (Worthington) as previously described [1], using 1200 units/ml. Digestion to determine the DNA repeat length was carried out at 20°C until 1–5% of the DNA was rendered acid-soluble, whereas the extensive digestion was done at 37°C until 40–55% of the DNA was converted to acid-soluble material. Digestion was terminated by addition of EDTA (2.5 mM final concentration). Aliquots were acid-precipitated in order to determine the amount of DNA which was rendered acid-soluble and the DNA was purified as previously described [1]. Rat liver digests, used as markers, were prepared as described elsewhere [1,14,15]. Polyacrylamide gels to determine the DNA repeat length were 2% acrylamide and 0.5% agarose and were run in an 11 cm long slab apparatus

[1]. After electrophoresis, gels were stained in 0.5 μg/ml ethidium bromide for 30 min and destained for 5 min. The gels were transilluminated with short wavelength ultraviolet light and photographed through a red filter. Gels were printed as negatives.

3. Results

Figure 1 shows a polyacrylamide gel with micrococcal nuclease digests of sea urchin sperm and gastrula cell chromatins. A rat liver digest is shown for comparison. It is clear that the DNA repeat of gastrule chromatin (slots 6–8) is longer than that of rat liver chromatin (slots 1, 5 and 9), but smaller than that of sperm chromatin (slots 2–4). A calibration curve (not shown) for the gel in fig.1 was constructed from the relative migration of the rat liver bands and their previously determined lengths in base pairs (see table 1 and ref. [14] and [15]). The mean size (center of the bands) of the sperm and gastrula DNA fragments was then obtained from this calibration curve (table 1). As previously noted [1,14,15] the length of the chromatin DNA repeat increases in the higher multiples until a plateau value is reached, due to exonucleolytic digestion. As shown in table 1, the lengths of the DNA repeats in sea urchin sperm and gastrula chromatins were 241 and 218 base pairs, respectively, when com-

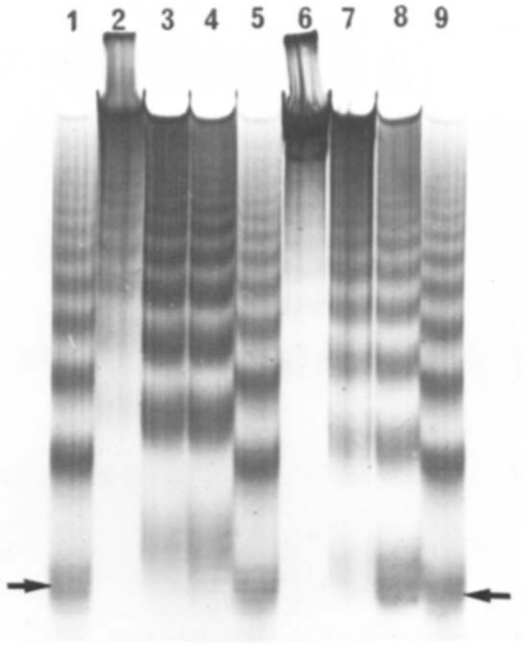

Fig.1. Polyacrylamide gel electrophoresis of the DNA of micrococcal nuclease digests of chromatins from sea urchin sperm cells (slots 2–4), sea urchin embryo cells at the gastrula stage (slots 6–8) and rat liver (slots 1, 5, 9). Nuclease digestions of sperm and gastrula chromatins were carried out for increasing times of digestion as described in Materials and methods. The arrows point to the position of the rat liver monomer DNA fragment.

Table 1
Length of the repeating unit of chromatins from sea urchin sperm and gastrula cells

Multiple	Chromatin					
	Rat liver		Sperm cells		Gastrula cells	
	Length of multiple	DNA repeat length	Length of multiple	DNA repeat length	Length of multiple	DNA repeat length
	base pairs		*base pairs*		*base pairs*	
Heptamer	1369	196	1681	240	1527	218
Hexamer	1176	196	1445	242	1304	217
Pentamer	980 (90)[a]	196	1207 (160)[a]	241	1089 (120)[a]	218
Tetramer	786 (91)	196	967 (161)	242	869 (119)	217
Trimer	583 (85)	194	722 (155)	241	645 (119)	215
Dimer	371 (70)	185	476 (137)	238	413 (109)	206
Monomer	183 (40)	-	228 (120)	–	191 (60)	–

[a]Numbers in parentheses indicate the width of the bands in base pairs.

The DNA lengths of the multiples were determined from the relative mobilities of the center of the bands (fig.1) as indicated in the text. The DNA repeat length was calculated by dividing the lengths of each multiple by the number of the band.

pared to the rat liver DNA repeat length of 196. These differences are not related to the method of nuclei preparation, since sea urchin gastrula and rat liver nuclei were prepared with the same method [12] and washing the gastrula nuclei with the buffers which were used for preparing sperm nuclei (see Material and methods) did not modify the length of the DNA repeat of gastrula chromatin (not shown). It it noteworthy that not only the mean length of the DNA repeat, but also the width of the bands increased from rat liver to gastrula chromatin and from gastrula to sperm chromatin (fig.1 and table 1).

Figure 2 shows that in spite of variations in the DNA repeat lengths, extensive digestion of rat liver (slots 1 and 5), gastrula (slot 4) and sperm (slots 2 and 3) chromatins with micrococcal nuclease produces a common pattern of DNA fragments smaller than the monomer repeat length. These fragments have the same size as those which were previously described in extensive digests of other chromatins of higher and lower eukaryotes (see Discussion).

4. Discussion

Our results confirm [24] that, like chromatins of other eukaryotic cells and in agreement with the model of Kornberg [16], the fundamental structure of chromatin of sea urchin sperm and gastrula cells consists of a basic repeating subunit. Electron microscopic studies have confirmed that the fundamental structure of sperm and gastrula chromatins is composed of a chain of nucleosomes (P. Oudet and C. Spadafora, unpublished results). In addition, the present results extend our previous observations [1] demonstrating that the length of DNA contained in the nucleosomes of higher eukaryotes is not invariable, ranging from 178 base pairs (CHO cells) to 207 base pairs (chicken erythrocytes). A similar high value has been found by Morris (personal communication) for the repeat length of the genetically inactive chicken erythrocyte chromatin. From our present results it appears that an increase in the DNA repeat length is not necessarily related to a repressed state of the genome, since sea urchin gastrula cells are very active in RNA synthesis. Taking into account the value (170 base pairs) which was recently found for the DNA repeat lengths of chromatin of lower eukaryotes [1,17], the length of DNA contained in the nucleosomes of a variety of eukaryote cells ranges from 170 to 241 base pairs.

Studies of extensive micrococcal digestion of chromatins from lower and higher eukaryotes have led to the proposal that the nucleosome contains a core consisting of two each of histones H2A, H2B, H3 and H4 and 140 base pairs of DNA which are more resistant to micrococcal digestion than the rest of the nucleosomal DNA [1,17–22]. The results shown in fig.2 indicate that extensive micrococcal digests of sperm and gastrula chromatins contain the same DNA band pattern as extensive digests of rat liver chromatin. In all cases, the largest and predominant submonomer DNA

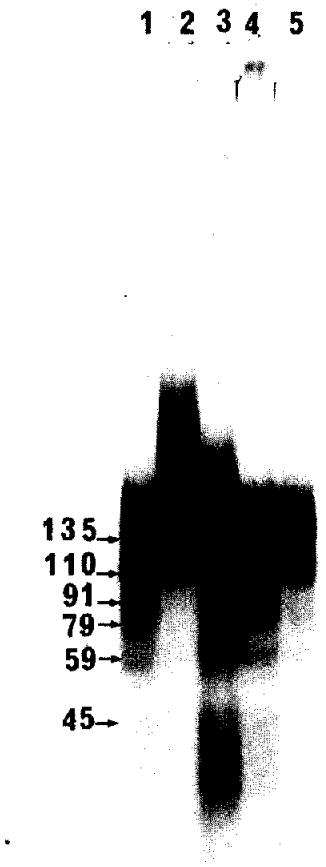

Fig.2. Pattern of DNA fragments generated by extensive micrococcal nuclease digestion. The digestion and the gels were carried out as described in Materials and methods. The gels were calibrated (in base pairs) as described in ref. [14] and [15]. Slots 1 and 5: rat liver digests used as markers (45% acid solubility); slots 2 and 3: sea urchin sperm digests (45 and 55% acid-solubility, respectively); slot 4: sea urchin gastrula digest (50% acid-solubility).

band was about 140 base pairs in length. Therefore, as for nucleosomes of other lower [17] and higher eukaryotes [1], the variability in the length of the DNA contained in sperm and gastrula cell nucleosomes appears to arise from variability in the length of the 'extra-core' DNA. From our present values the 'extra-core' DNA would amount to 56, 78 and 101 base pairs for rat liver, sea urchin gastrula and sperm nucleosomes, respectively. That most of the 'extra-core' DNA is equally susceptible to micrococcal nuclease attack is suggested by the maximum widths of the DNA bands (fig.1 and table 1) which in the three cases are directly related to the lengths of the 'extra-core' DNA.

As mentioned in the Introduction, the origin of the variability of the DNA length contained in the nucleosomes could be related to the existence of different histone cores containing different variants of histones H2A and H2B. It could also be related, as it was proposed by Noll [17], to variability in histone H1. Since sea urchin sperm and gastrula cell chromatins appear to contain different variants of all three histones (see Introduction), our present study does not distinguish between these two possibilities. Chromatin reconstitution experiments [23] by associating the DNA and the isolated histones should help to solve the problem or the origin of the variability of the DNA lengths contained in the nucleosomes. However, our present results exclude acetylation of histone H3 and H4 as a possible source of the variability. The DNA repeat lengths of chicken erythrocyte chromatin which contains non-acetylated H3 and H4 histones [8] is longer (207 base pairs, ref. [1]) than that of rat liver chromatin, but smaller than that of sea urchin gastrula chromatin, both of which contain multiacetylated forms of H3 and H4 histones [8].

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Note added in proof

N. R. Morris [Cell, 8 (1976) 357–363] and J. O. Thomas and V. Furber [FEBS Lett., 66 (1976) 274–280] have recently found that the DNA repeat length of *Aspergillus nidulns* and yeast chromatins are 154 and 165 base pairs, respectively.

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